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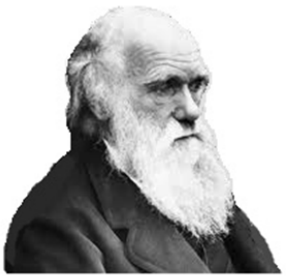

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Bdellovibrio and like organisms: The much-anticipated "magic bullet"

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ABSTRACT

With advances in next generation sequencing and microscopy, a clearer picture is beginning to emerge about the predatory properties of *Bdellovibrio* and like organisms (BALOs). BALOs are Gram negative microorganisms which are natural predators of other Gram-negative microorganisms, especially those associated with diseases in humans and animals. One of the limitations of BALOs is their inability to prey upon Gram-positive organisms that cause the bulk of human diseases. The global spread of antibiotics resistance to almost every group of antibiotics, and the paucity of newer antibiotics since 1970s is very worrisome. The ability of BALOs to decimate pathogen populations principally those carrying multidrug résistance genes coupled with the increasing rate of antibiotics resistance, has made them an attractive option as biocontrol agents. With the absence of resistance to BALOs, these much anticipated "magic bullets" will certainly find more and more applications in health, agriculture, medicine and environment in the nearest future.

***Keywords*:** *Bdellovibrio*, Predation, Antimicrobial agent, Resistance, biocontrol agents

1. INTRODUCTION

In the microbial world, prokaryote and eukaryote predation have emerged as a factor capable of shaping microbial structure and as well as diversity [1-2]. The most studied microorganisms that exhibit direct or indirect contact predation are members of the class Deltaproteobacteria specifically those of *Bdellovibrio* and like organisms (BALO) [3, 4]. In

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addition to *Bdellovibrio*, predation in the microbial world is also encountered in a few other genera and these include *Bacteriovorax*, *Bacteriolyticum* and *Peredibacter* [5]. However, *Bdellovibrio* remains the most studied genera.

Bdellovibrio is an important predator that has evolved special abilities to prey on other bacteria especially the Gram-negative (GN) counterpart. *Bdellovibrio* was first isolated and described in 1962 by Stolp and Petzold while they were attempting to isolate bacteriophage from soil [6]. As microorganisms, it is ubiquitous in nature and has been isolated from various environments such as sewage, soil, sediment, mammalian intestine and fresh water [7-9]. The genus *Bdellovibrio* is generally known for their predatory abilities on other GN bacteria including the pathogenic species [3, 4]. *Bdellovibrio* are vibroid-like in morphology and measure about 0.3 to 0.5 by 1.4 to 2.5 μm in dimension [6. It is highly motile via an active single polar flagella [1, 3].

It belongs to the order Bdellovibrionales and family Bdellovibrionaceae [3, 4]. Knowledge of the mechanism of its predation lifestyle is still limited. It utilizes two stages: free-swimming stage (the attack phase) and the growth phase [10]. Furthermore, molecular studies have revealed genome size higher than those of common intracellular parasites [11].

A recent study revealed a number of interesting and promising properties. These include the absence of toxicity, inflammatory immune response and cell detachment on human keratinocytes, human liver epithelial cells, human kidney epithelial cells, loosely adherent human spleen monocytes and suspension of human blood monocytes [12]. These properties have attracted a number of potential applications in medicine, and as well as agriculture, and environment. Its potential applications in medicine seems enormous especially with the rapid spread of antimicrobial resistance to almost every class of available antibiotics by microorganisms which habour multiple antibiotics resistant genes [13]. The aim of this review is to briefly describe the cellular properties, life cycle, target species, application and future prospects of *Bdellovibrio* and like organisms.

2. CELLULAR FEATURES

Bdellovibrio bacteriovorus (ATCC® 15356™) deposited by Stolp and Starr [6] and as enumerated by Chatterjee [11] and Sinha [15] have the following properties listed below in the table and figure.

Table 1. Cellular properties of Bdellovibrio.

S/N	Properties
Gram reaction	Negative
Cell dimension	0.2-0.5 μm wide and 0.5-2.5 μm long
Predatory organisms	Gram negative
Shape of cell	Curved vibrio-like
Arrangements	Singles

Endospore formation	No
Motility	Yes (uniflagellate and sheathed)
Oxygen requirement	Aerobic
Habitat	Ubiquitous
Optimum temperature	28-30 ºC
Temperature Range	Mesophilic
Biosafety requirement	Level 1
Duration of predatory life cycle	34 hours
Prey range	Wide

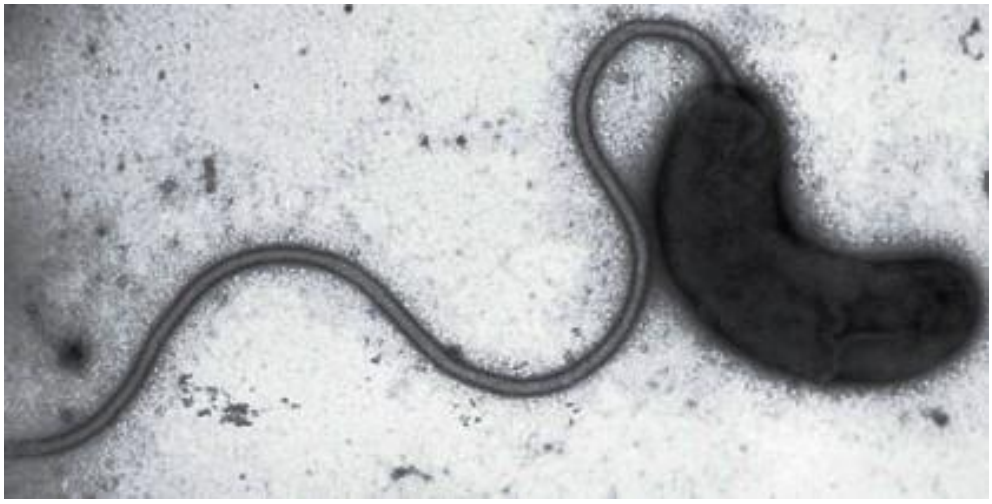

Figure 1. *Bdellovibrio bacteriovorus* structure [15].

3. TARGET SPECIES

Bdellovibrio is an interplasmic natural predator of Gram-negative organisms and it favourite preys include *Escherichia coli* and *Pseudomonas aeruginosa* [16]. One of the interesting and exciting applications of BALOs is their efficient predation of multidrug resistant clinical pathogens [17]. In a previous study, it was shown that *B. bacteriovorus* can reduce and also prevent the formation of biofilms by multidrug and extensive drug resistant *E. coli, K. pneumoniae, P. aeruginosa*, and *Acinetobacter baumannii* [17]. Other prey of BALOs includes *Salmonella* species [18]. Although BALO naturally preys on mainly Gram-negative organisms; however, evidence available indicates it can reduce biofilm formation in *Staphylococcus aureus* [16].

4. HABITATS AND ABUNDANCE

Predatory behaviour exist in atleast four different bacterial phyla namely Actinobacteria, Bacteroidetes, Chloroflexi, and Proteobacteria. Representative species in these phyla deploy different strategies for predation [19, 20]. The arrival of culture dependent techniques such as metagenomics have made available a number of microbial structures and functions previously out of reach to become more and clearer [21, 22]. Using metagenomics analysis, several predatory bacterial taxas especially amongst the Bacteroidetes, Deltaproteobacteria and Cyanobacteria have been discovered. Altogether, predatory organisms made up 0.5% of the bacterial community in their sampled environment [20].

First isolated from soil in 1962 [6], *Bdellovibrio* have now been isolated from a number of habitats. Chu and Zhu isolated the bacterium from cultured fish ponds using *Aeromonas hydrophila* J-1 as host [23]. In another study using various Gram-negative hosts, *Bdellobvibrio* was isolated from fresh water samples [9]. Other habitats they have been isolated from include sewage, sediment, and mammalian intestine and fresh water [5, 7, 8]. Feng *et al* [2] isolated *Bdellovibrio* from activated sludge floc and granules and further showed that they significantly affected microbial communities (> 90%) within 24 hours. Recently, it has been shown to also abound in the human gut in different proportions in disease and health [24].

5. GENOME AND PROTEOME COMPOSITION

Next generation sequencing (NGS) technology has been used by several authors to evaluate their proteomic and genomic compositions [4, 7, 25, 26]. Chatterjee [11] revealed that *B. Bacteriovorus* HD100 has a larger genome when compared to other obligate intracellular parasites such as *Rickettsia prowazekii, Chlamydia trachomatis, Chlamydia pneumoniae, Mycobacterium leprae, Rickettsia prowazekii and Rickettsia conorii* [27,28]. Habitat is strongly linked to genome sizes amongst the parasites and is also function dependent [28]. *B. Bacteriovorus* HD100 possesses a genome size of 3782950bp. In addition, it has an overall GC content of 50.70% and GC content in coding region of 50.40%. Other genomic properties include open reading frame of 3584 and coding sequences of 1995. It has a number of hypothetical proteins (1207) and hydrolytic enzymes distributed as thus 150 proteases, 200 DNases, 9 RNases, 15 lipases, 10 Glycanases and 89 others [11]. *Rickettsiae prowazekii* genome is 1.1mbp and has a G+C content (29%), almost twice as smaller than that of *B. Bacteriovorus* HD100 [27]. This low G+C content suggest a high spontaneous deletion rate and mutation bias towards A+G. In strain HD100, the abundance of hydrolytic enzymes shows why these organisms are an effective predator. Their large genome size is attributed to their unique life cycle [1]. Hobley *et al* [25] compared the genome of Tiberius isolated and that of HD100 strain and it revealed some interesting genomic details (See Figure 2). First, self-recognition does occur amongst the *Bdellovibrio* species. Second, there is significant conservation between both species and finally, evidence exist for lateral gene transfer. Earlier, analysis of the proteome and genome composition showed prey derived horizontal gene transfer [4]. Recently, whole genome sequencing and comparisms of two *Bdellovibrio* spp. isolated from soil was carried out [28]. Their findings revealed distinctive genes that encode hydrolytic, chemotaxis, and transporter proteins which have been previously implicated in predation [1]. In addition, they showed that both strains differed in terms of G + C and amino acid compositions, and 16S rRNA gene sequence.

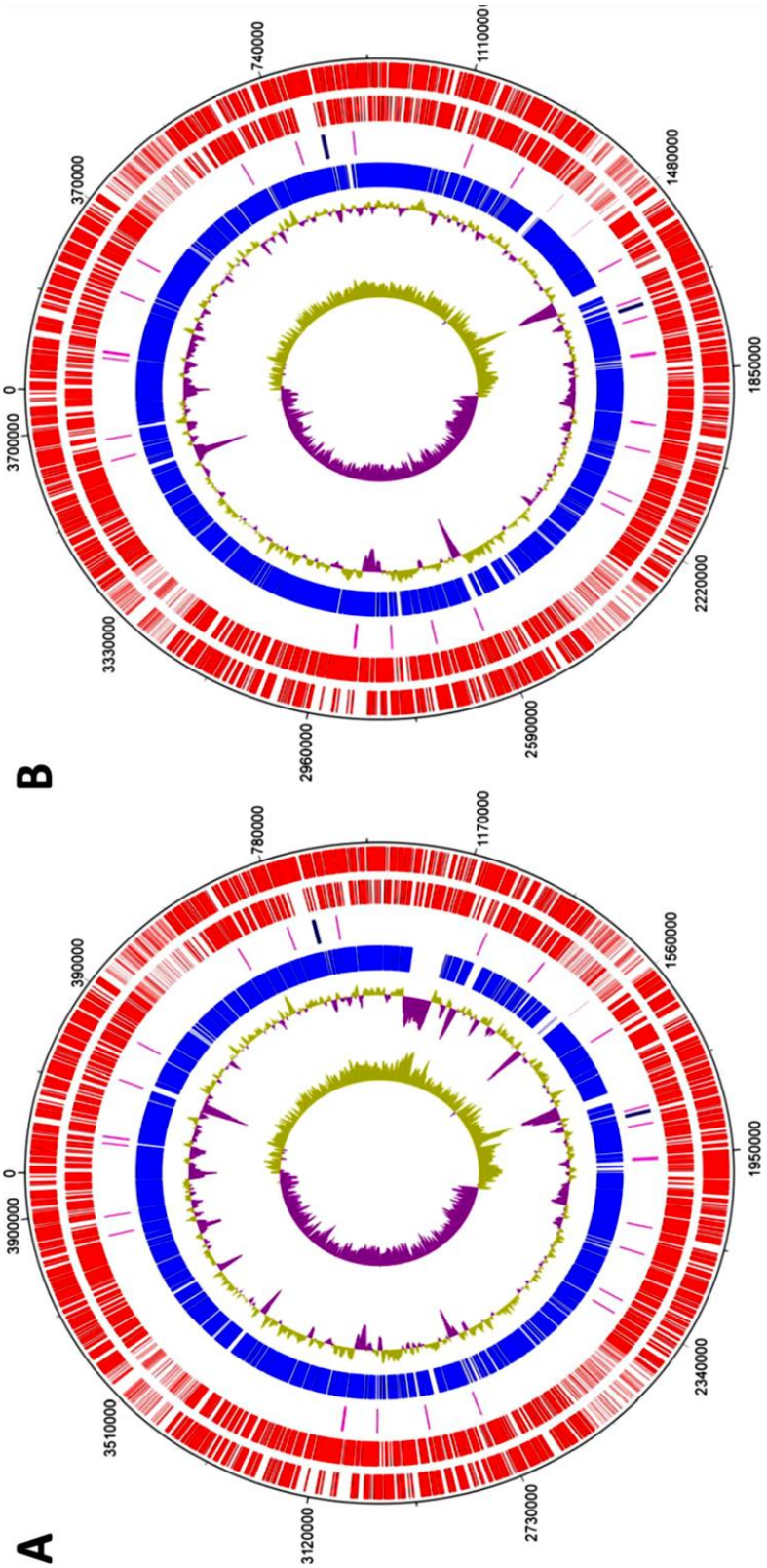

Figure 2. Whole genome comparisms of B. bacteriovorus (A) Tiberius and HD100, (B) strains using a DNA plotter [25].

Wurtzel *et al* [30] showed that NGS can capture mutated genes in wild type and mutant species. *Bdellovibrio bacteriovorus* and *exovorus* have been shown to have same number and types of peptides and amino acids uptake systems and secretory systems [31].

6. LIFE CYCLE

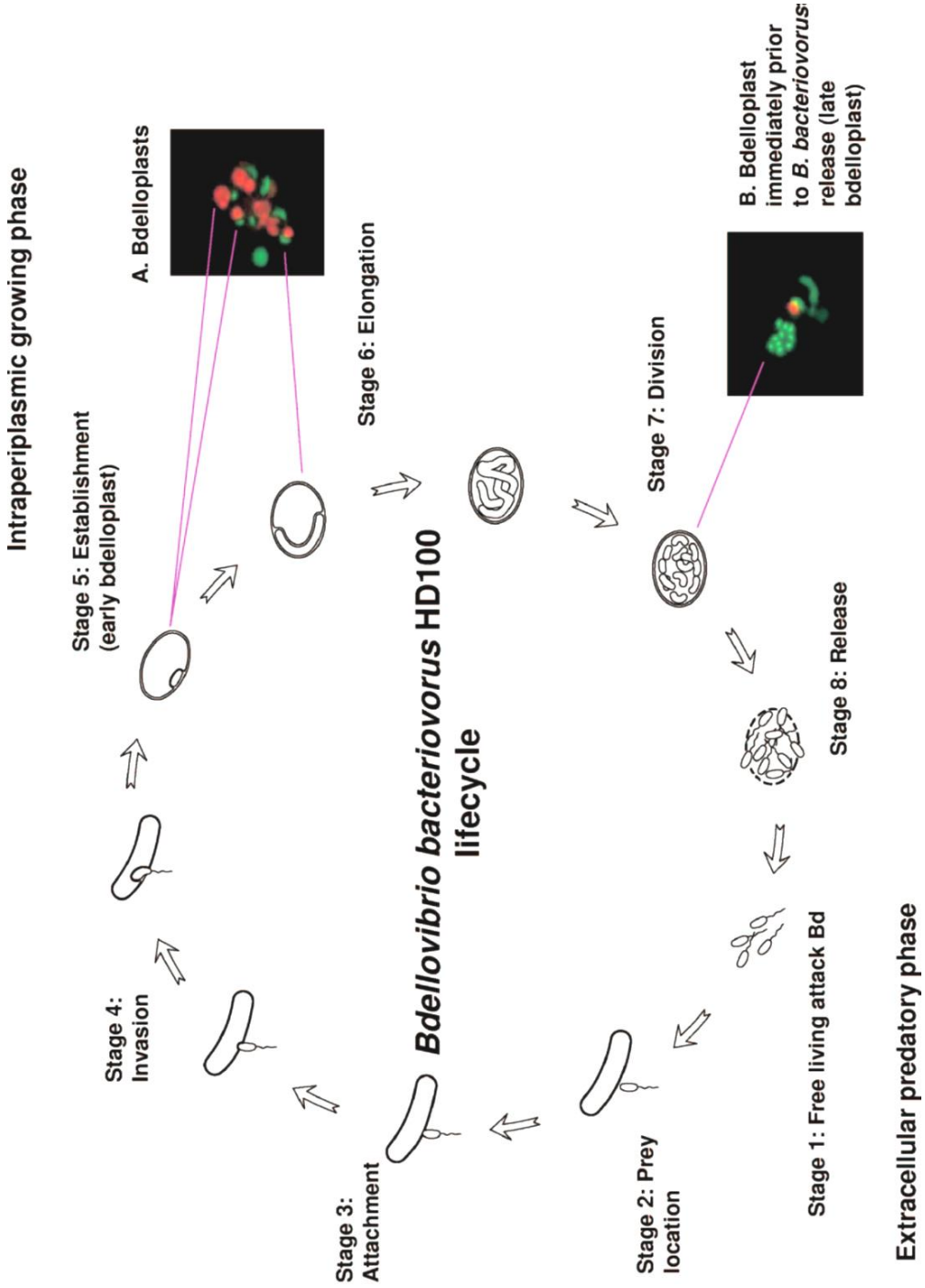

Figure 3. Life cycle of *B. Bacteriovorus* [32].

The life cycle of BALO is most studied in *Bd. Bacteriovorus* which uses the periplasmic life cycle that is divided into two stages namely a free-swimming (the attack phase) and the growth phase [32, 33]. The free-swimming phase is also called the extracellular predatory phase [32]. Both phases are composed of 8 stages as shown below in Figure 3. The phases are free living attack *Bd*, location of a suitable prey, attachment to prey, invasion, establishment (early bdelloplast), elongation, division and release of progeny. Upon release of the progenies, finding a prey as soon as possible is key for its survival [1]. Finding a prey is made possible because *Bdellovibrio* and like organisms possess a polar flagella [3]. Earlier studies showed that chemotaxis plays a role in finding a suitable prey [34, 35]. Higher density of prey increases the chances of prey attachment [1]. Recently, it has been shown that *B. Bacteriovorus* uses two unidentified prey-derived signals that are spatially and temporary separated to drive prey recognition and predatory growth [36, 37]. Otto *et al* [38] showed evidence that hyphae enhance the accessibility of *Pseudomonas fluorescens* LP6a to *B. bacteriovorus* 109J. Suitable attachment to a prey envelope is preceded by a reversible and non-specific recognition period by BALO. Where the preyed cell is Gram-negative, the attachment becomes irreversible [39]. Varon and Shilo [39] maintained that temperature, pH and medium composition are factors that drive attachment. Flagellar shedding is important in the stages after attachment even though it still not clear when this is done [1, 40], hydrolysis and not flagellar rotation is needed in invasion. Chanyi [1] showed retraction of type IV pili using PilT1 and PilT2 mutants after attachment. Gene expression studies have shown that hydrolytic enzymes are upregulated during initial attachment of the predation [41].

When inside the periplasmic space, the entry pore is sealed and eventually a bedelloplast is formed. Its formation is very crucial for the growth of the BALO, indicates successful attack and the start of the growth phase. Inside this structure, growth conditions are optimised; it is osmotically stable and fends off further BALO attacks [1].

Lambert *et al* [42] and Karunker *et al* 41] using microarray technique and RNA-seq analyses identified a number of genes expressed by B. *bacteriovorus* growth. Upregulated hydrolytic enzymes are prominent in the degradation of prey macromolecules [1]. Septation and progeny release is still not fully understood. However, Fenton *et al* [43] showed that release of progeny is via pores in the bdelloplast.

7. POTENTIAL APPLICATIONS

The potential applications of *Bdellovibrio* in health, environment and agriculture are endless. The medical applications are very promising especially with a recent study showing absence of pathogenicity, cytotoxicity, cell detachment and inflammatory cytokines on five different human cell lines [12]. Briefly, these are as discussed below.

8. FUTURE ANTIMICROBIAL AGENT

Starting from the 1970s, there has been a drastic decline in the number of antibiotics that have made it to the market [44]. Furthermore and worrisomely, in the last three decades, there has been no registered class of new antibiotics [44]. Reports indicate that microbes have acquired resistance to even colistin, the so called “drug of last resort” [45]. Antibiotic resistance and the emergence of antibiotic resistant pathogens have assumed a global dimension [46]. Clearly, it has outpaced new antibiotics development and this scenario has prompted the need

to search for newer antibiotics. Microorganisms that are resistant to antibiotics abound in the environment and are endowed with various evolutionary and survival mechanisms with which they spread and acquire resistance genes [13, 47-48]. These genes have been detected in various isolates and environmental samples including clinical wastes and effluents using various techniques [46-49]. Even more worrisome is their capacity to be transferred to isolates in the clinical settings [46, 49]. Medicinal plants have received quite some attention as they contain various phytochemicals that have been linked to their antimicrobial activities. As expected, a number of researches have emerged that have shown the presence of these phytochemicals in plants and as well their *in-vitro* activities against various multi-drug resistant clinical and environmental isolates. Most of the studies have utilized crude extracts and they showed and even amongst these extracts microorganisms show various degrees of sensitivity while some are even resistant [50-54].

One of the attractive features of *Bdellovibrio* is its predation of pathogens associated with human diseases [17, 55-60]. Investigators have shown how *B. bacteriovorus* and *M. aeruginosavorus* preyed on isolates harbouring various resistant genes notably extended-spectrum β-lactamase, KPC-type carbapenemase, AmpC-type β-lactamase, metallo- β-lactamase, and as well as fluoroquinolone resistant *P. aeruginosa* ocular isolates. Sun *et al* [17] showed predation against multidrug-resistant (MDR) or extensive drug resistant (XDR) gram-negative pathogens and their associated biofilms. Interestingly, in their study, the most efficient predation was against *E. coli* and reductions in biofilms formation were 65.2%, 37.1%, 44.7%, and 36.8% for *E. coli, K. pneumoniae, P. aeruginosa*, and *A. baumannii,* respectively. In a recent study, Dharani *et al* [61] showed that *A. baumannii* ATCC17978, *Escherichia coli* ATCC 25922, *K. pneumoniae* ATCC13883 and *P. aeruginosa* ATCC 47085 harbouring colistin resistant gene mcr-1 on recombinant plasmids pMQ124-mcr-1 were susceptible to predation by *Bdellovibrio bacteriovorus* and *Micavibrio aeruginosavorus*. BALO have recently been used in the treatment of acute hepatopancreatic necrosis disease, a severe disease of shrimp, caused by *Vibrio parahaemolyticus* [8]. In their study, they observed reduction in mortality and biofilm formation by *Vibrio parahaemolyticus* by BALO.

9. EFFECT ON HUMAN AND ANIMAL MICROBIOME

The use of *Bdellovibrio* is very attractive and promising for a number of reasons. Interestingly and importantly, there is no report thus far that indicates that prey organisms become resistant to *Bdellovibrio* and this makes BALO very attractive as biocontrol agents. Studies thus far have shown that *Bdellovibrio* administered via injection to tissues using living animal models are safe and not pathogenic [18, 62]. Human microbiome is very complex and is strongly implicated in health and disease [22].

Atterbury *et al* [18] showed that orally administered predatory *Bdellovibrio* to birds predosed with gut-colonizing *Salmonella enterica* serovar Enteritidis phage type 4 strain reduced the *Salmonella* population in their gut and also abnormal fecal morphology. Using rat lungs, Shatzkes *et al* [62] showed that *Bdellovibrio* aerosol reduced the burden of *Klebsiella pneumoniae*. In another study that utilised Zebra fish larvae as animal model, Willis *et al* [62], revealed significant synergy between host immunity and *Bdellovibrio* predation activity against *Shigella flexneri* infection. In their *in vivo* study, *Bdellovibrio* reduces *Shigella* load to a level where the host immune cells can clear and this lead to host survival. In humans, studies abound that provide evidence that *B. bacteriovorus* is a part of the human gut microbiome [64, 65]. A

recent study has shown a relationship between decreased population of *Bdellovibrio* in human subjects and predisposition to inflammatory bowel diseases, celiac disease and cystic fibrosis compared to higher abundance in healthy subjects [65]. In a more recent study, inoculation of rats with *Bdellovibrio* revealed minimal shifts in taxonomic groups a week after inoculation [62]. Both studies suggest suggesting that their presence in the gut may contribute to health.

Oral health

Two main diseases affect oral health and these are dental caries, also called tooth decay and periodontal or gum disease. Periodontal disease is caused by inflammatory response to bacterial biofilm formed along the gum line leading to damage to tissue and bones that surround the teeth. Studies have shown that *Bdellovibrio* could attack and prey on Gram-negative organisms implicated in periodontal disease [66, 67]. In another study, BALOs have been shown to access biofilms formed in periodontal health [15]. Sinha *et al* [15] suggested that it could be used as an adjunct for local drug delivery as it is unaffected by the β-lactam antibiotics.

Agriculture

One of the driving forces of antibiotics resistance is their indiscriminate usage in agriculture to control microbial diseases amongst livestock such as those caused by *Salmonella* species [68]. Recently, the U. S. Food and Drug Administration reported recall of over a billion eggs contaminated with *Salmonella enteritidis*. A study by Atterbury *et al* [18] revealed significant reduction in *Salmonella* reduction with no side effect in birds administered an oral dose of predatory HD100 *Bdellovibrio.* Although more studies on safety are needed, these early findings hold a lot of promise and could eliminate the extensive use of antibiotics. In an earlier study, *Bdellovibrio bacteriovorus* 109J was able to lyse 32 food spoilage and food borne pathogenic bacterial strains [69]. *Bdellovibrio* show promise in controlling plant pathogens. However, studies evaluating their effect on plant pathogens are very few [36]. One of the challenges facing food security especially in third world countries is that of post-harvest losses. BALO have been used to eradicate *Xanthomonas oryzae* [70]. Jurkevitch *et al* [71] isolated 13 soil and 7 common rhizosphere *Bdellovibrio* species using *Pseudomonas corrugate* as prey.

Source of antibiotics

Predatory microorganisms abound in the environment where they act singly or in packs as predators. The predatory strategy of the latter involves the use of antibiotics and this is consistent with genome sequencing studies [19]. Genomic sequences reveal a link between secondary metabolism and predation. Important genera in this regard include *Myxobacteria* and *Herptosiphon*. For details on the various natural with antibiotics potentials produced by these microorganisms' readers are referred to Korp *et al* [19].

Biological cleaning agent for membrane bioreactor systems (MBRs)

Membrane bioreactors are the technology of choice for waste water treatment [72]. However, one major challenge of MBRs called biofouling and it reduces its efficiency greatly [73]. Yilmaz *et al* [74] showed that treatment of membrane with *B. bacteriovorus* caused significant improvement in membrane flux and efficiency amongst others.

Wounds healing

Chronic wounds such as diabetic ulcer of the foot pose a significant global health care challenge [75]. As expected, a number of therapies have emerged around the world. These include hyperbaric oxygen, negative wound pressure, biophysical, growth factors, acellular matrix tissues, bioengineered allogenenic cellular therapies and stem cell therapies [75]. However, studies assessing their efficacy are lacking [75]. One major impediment to wound healing is that of infection caused by antibiotics resistant bacterial strains [76, 77]. Furthermore, honey have been shown to be effective against partial thickness burns in less than a week compared to conventional dressing. However, there is evidence to show that it is an effective antiseptic when used in infected surgery wounds [78]. The ability of BALO to predate on Gram negative bacteria isolates implicated in chronic wounds will be the driving force for research into this area.

Potential drawbacks and prospects

Although *Bdellovibrio* have been shown to be non-pathogenic on animal models, the main concern still remains that of safety given that they encode about 3,500 gene products. Thus far, there is no study that has shown that *Bdellovibrio* have been introduced on human subjects and the idea may sound so invasive and unconventional [46]. Furthermore, bacteria with an S-layer on their surfaces are resistant to BALOs and it seem that BALOs do not completely kill them even at a higher predator to prey ratio. Compared to antibiotics resistance, the resistance is lost quickly and have not been shown to spread [15, 79, 80]. Another limitation that could potentially limit their use is the fact that BALOs appear to be strict aerobes based on previous studies and this could lower their use in reduced oxygen and anaerobic environments such as in the gut, urinary tract and periodontal cavities [15, 80]. However, recent findings suggest that these organisms could survive long enough and attack in low oxygen environments, and also elaborate nitric oxide and nitrate reductase. Their activity appears to be affected by chemicals and pH. Glucose and glycerol in high concentration have been shown to affect the activity of BALOs. Interestingly, their activities are not affected by the beta-lactam, and as suggested by Dwidar *et al* [80], it can be used side by side with antibiotics in clinically setting especially in situations where causative pathogens are those with multi-drug resistance genes. Furthermore, their predatory activity thus far is limited only to human pathogens that are Gram-negative. However, most human pathogens such as *Staphylococcus aureus* that is so often associated with nosocomial infections and often displaying multi-drug resistance is a Gram positive [15]. From the foregoing, it is obvious that BALOs will play a significant role as far as the search for newer and safer antibiotics is concerned. Studies on the predatory effects of BALOs on urinary tract pathogens and those responsible for neonatal sepsis will certainly emerge.

10. CONCLUSION

Pathogens with multi-drug resistant genes are fast becoming a public health menace around the world. *Bdellovibrio* and like organisms holds a lot of promise due to its predatory nature toward Gram-negative organisms despite drawbacks. Owing to the unique life style of this organism, genetic manipulations that will hasten the numerous potential applications is urgently needed.

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